

RELATIONSHIP BETWEEN ALLELES OF GENE BOLA-DRB3 AND SOMATIC CELLS AMOUNT IN MILK OF UKRAINIAN BLACK-AND-WHITE DAIRY BREED

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*A significant genetic correlation between the indicator of the somatic milk cells amount (SCC) and intramammary infection has been established, making it possible to use it in breeding programs for reducing of mastitis in cows. Various studies demonstrated that resistance (susceptibility) to mastitis is genetically predetermined. Among the genes associated with diseases, special attention is paid to exon 2 of the gene BoLA-DRB3, which is quite polymorphic. The aim of the study was to identify associations between alleles of the gene BoLA-DRB3 and SCC among cows of Ukrainian black-and-white dairy breed (N = 92). The RFLP-PCR method revealed 31 BoLA-DRB3.2 alleles. The relative risk index and the χ^2 test were used to establish a link between the allele frequencies and the SCC. Two alleles with strong association with low level of SCC (CI = 0.95): BoLA-DRB3.2*22 (P(A) = 0.06; RR = -3.43; χ^2 = 3.84) and BoLA-DRB3.2*28 (P(A) = 0.076; RR = -4.14; χ^2 = 6.17) were revealed. An additional check using the precise Fisher's exact test controlled by Pearson's contingency coefficient showed that only allele BoLA-DRB3.2*28 can be used as a marker in connection with low SCC in cows of Ukrainian black-and-white dairy breed. The allele *28 as a SCC marker was revealed for the first time. Analysis of previous studies shows discrepancies between the results that link different BoLA-DRB3.2 alleles with SCC, obtained for different breeds and even within the same breed. In future, it is necessary to expand the research by increasing the research sample and determine the associations between the number of somatic cells not only in connection with allelic polymorphism, but also in connection with the genotypes of the BoLA-DRB3 gene. Interstitial application of the new antibacterial liposomal preparation has led to a decrease in the number of somatic cell cultures in the afflicted animals with subclinical mastitis of cows.*

Keywords: COW, ALLELE, GEN, MASTIT, SOMATIC CELLS

ЗВ'ЯЗОК МІЖ АЛЕЛЯМИ ГЕНА BOLA-DRB3 ТА КІЛЬКІСТЮ СОМАТИЧНИХ КЛІТИН У МОЛОЦІ УКРАЇНСЬКОЇ ЧОРНО-РЯБОЇ МОЛОЧНОЇ ПОРОДИ

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*Між показником кількості соматичних клітин молока (КСК) та інтрамамарною інфекцією встановлено значну генетичну кореляцію, що дозволяє використовувати його в селекційних програмах зниження захворюваності корів на мастит. Різні дослідження довели, що резистентність (сприйнятливість) до маститів визначається генетично. Серед генів, пов'язаних із захворюваннями, особливу увагу приділяють екзону 2 гена BoLA-DRB3, який є досить поліморфним. Метою дослідження було виявлення асоціацій між алелями гена BoLA-DRB3 і КСК серед корів Української чорно-рябої молочної породи (N = 92). Методом ПДРФ-ПЛР виявлено 31 алель BoLA-DRB3.2. Для встановлення зв'язку між частотами алелів та КСК використано показник відносного ризику та тест χ^2 . Виявлено два алеля, які мають сильну асоціацію з низьким рівнем SCC (CI = 0.95): BoLA-DRB3.2*22 (P(A) = 0.06; RR = -3.43; χ^2 = 3.84) і BoLA-DRB3.2*28 (P(A) = 0.076; RR = -4.14; χ^2 = 6.17). Додаткова перевірка за точним критерієм Фішера з контролем за коефіцієнтом спряженості Пірсона показала, що тільки алель BoLA-DRB3.2*28 може використовуватись*

як маркер у зв'язку з низьким КСК у корів Української чорно-рябої молочної породи. Алей *28 як маркер КСК виявлено вперше. Аналіз подібних досліджень показує розбіжності між результатами, що зв'язують різні алелі BoLA-DRB3.2 з КСК, отриманими для різних порід і навіть в межах однієї породи. В подальшому необхідно розширити дослідження шляхом збільшення дослідної вибірки та проводити визначення асоціацій між кількістю соматичних клітин не тільки за алейним поліморфізмом, а й за генотипами гена BoLA-DRB3. Інтрацистернальне застосування нового антибактеріального ліпосомального препарату призвело до зниження кількості соматичних клітин молока у хворих на субклінічний мастит корів.

Ключові слова: КОРОВА, АЛЕЙ, ГЕН, МАСТИТ, СОМАТИЧНІ КЛІТИНИ

Mastitis is the most common breast disease that causes maximum damage to the dairy livestock. It is a complex problem because it is caused by many factors acting at the same time. One of the methods to decrease mastitis in dairy cows is genetic breeding.

The most suitable parameter for indirect selection based on phenotype for reducing of mastitis was the number of somatic milk cells (SCC). Many countries have incorporated a SCC research program as a way to improve resistance to intramammary infections.

Milk somatic cells include 75 % leukocytes, i.e. neutrophils, macrophages, lymphocytes and erythrocytes and 25 % epithelial cells [18]. Small amounts of somatic cells, usually presented in milk, try to immediately eliminate intramammary infection. During bacterial pathogenesis, macrophages are used to facilitate the natural or adaptive immune response. Like neutrophils, nonspecific functions of macrophages lie in phagocytosis of pathogenic bacteria and destroy their protease and active radicals of oxygen. Epithelial udder cells can play a protective role in the prevention of infection due to absorption and digestion of microbes [27].

In most of genetic researches SCC and clinical mastitis are considered to be phenotypic signs for forecasting of bacterial insemination of an udder [8]. The genetic correlation between SCC and bacterial infection is estimated close to one, indicating that somatic cells and subclinical infections are essentially one sign [32]. Most of the estimations of the genetic correlation between SCC and clinical mastitis fluctuate from 0.5 to 0.8, on average 0.7. The high correlation values indicate that the SCC and clinical mastitis are partial cases of signs that include the same genes [22].

Due to the limited progress in improving the health of the udder by conventional selection procedures using indirect signs, the demand for molecular markers regarding resistance to mastitis

was increased. Researchers focused on identifying more informative genetic markers that allow faster and more accurately select cattle, resistant to mastitis. Among the genes associated with the decrease in frequency of mastitis, particular attention is paid to the BoLA-DRB3 gene due to its special role in the immune system [25].

The products of BoLA complex participate directly in the binding of alien antigens and determine the specificity of the immune response. The BoLA-DRB3 gene encodes the β -chain of molecules of the Class II MHC, involved in the antigen presentation on antigen-presenting cells. It is usually expressed by cells of the immune system, such as macrophages, dendritic cells that treat the antigen and then represent it to the T helper to initiate an immune response. BoLA-DRB3 is the most polymorphic genome of the major histocompatibility complex of cattle [17, 27].

Somatic cells are constantly present in milk. Their high concentration is a sign of milk secretion violation or diseases. Therefore, the level of SCC is a sanitary indicator of the quality of raw milk collected and animal diseases. Normal cow milk has a regular level of SCC 100–150 thousand cells/ml. Higher numbers indicate secretory disorders and diseases. The increase in the content of the somatic cells over 200 000 in 1 ml of milk indicates the infection of an udder of infectious agent [4, 21].

When treating the afflicted cows, in most cases preference is given to the use of antibiotics and sulfanilamide drugs through intracysternine administration. The most negative consequence of the use of antibiotics in the treatment of mastitis of cows is the presence of their residues in pre-packed milk, worsening its technological properties and harming people's health [19].

Therefore, in recent years, scientific research on the use of liposomal drugs, which do not contain antibiotics, contribute to the prevention of relapse of the disease, and the maximum

restoration of milk production, has significantly expanded. Liposomes are derived from natural lipids, therefore they are non-toxic, do not cause unwanted immune reactions, are subject to biological degradation, that is, they are destroyed by the action of conventional enzymes present in the body and therefore they can be considered ideal drug carriers.

The research used the “Limanin” liposomal drug developed in the immunology laboratory of the Institute of Animal Biology of the National Academy of Sciences, which contains an extract from the St. John’s wort, vitamins, lecithin, and tween.

The aim of this study was to assess the suitability of the BoLA-DRB3 gene polymorphism for determining the phenotypic value of the somatic cells amount (as a measure of udder health) of Ukrainian black-and-white dairy livestock and to find out the effect of the “Limanin” drug on the number of somatic cells in the milk of cows suffering from the subclinical form of mastitis.

Materials and methods

The research was carried from 2015 to 2018 years out in breeding farm LLC “Kozatska Dolyna 2006” of the Khmelnytsky region engaged in breeding Ukrainian black and white dairy breed. The samples venous blood taken from 92 cows we investigated in the Genetics Laboratory of the Institute of Animal Breeding and Genetics of the National Academy of Agrarian Science of Ukraine.

A study of the herd in order to detect resistant and sensitive cows to mastitis was conducted on a monthly basis. The research was carried out on special milk control plates with four holes, in which 1 ml a milk of each quarter udder and an equal amount of reagent was added. With a positive reaction, a clot of jelly-like consistency is formed. Such a reaction indicates the presence in milk of at least 500 thousand somatic cells per 1 ml. Clinical mastitis were determined during milking. Identification of the disease based on proportionality quarters udder, pain sensitivity, increase local and general temperature, swelling, seal the udder, secretion (the impurity of blood, pus, change the color, consistency).

Sampling of milk and delivery to the laboratory was carried out in accordance with DSTU ISO 707:2002 [15]. Determination of the number of somatic cells performed directly calculation by Prescott-Breed’s method [2].

The alleles frequencies we revealed based on the polymorphism analysis of the length of the restriction fragments (PCR-RFLP) of the products of amplification of the exon 2 of the BoLA-DRB3 gene [29]. DNA’s isolation from blood were performed out using *DIAtom-TMDNA Prep200* kits (*Isogen Laboratory Ltd.*). DNA isolated from fresh biological material has high molecular weight (40–50 bp) and pure substance. The concentration and purity of extracted DNA were assessed by spectrophotometry and electrophoresis in 1 % agarose gel. With this purpose, 25, 50, and 100 ng of λ phage DNA and aliquots of solution with an unknown concentration applied to agarose gel. Electrophoresis was performed in 1x Trisborate (TBE) buffer (89 mM Tris-OH, 89mM H_3BO_3 , 2mMEDTA) with EtBr (1 $\mu\text{g}/\text{ml}$) at constant voltage of 120 V. The concentration of DNA of the test samples were determined by comparing the fluorescence intensity of the aliquots from solutions of unknown concentration and control λ phage DNA.

The BoLA-DRB3 exon 2 was amplified by PCR using PCR as modified from Van Eijk [31]. The PCR was carried out using ready-made sets of “GenPakR PCR Core”, LLC “Izogen Laboratory” (Russia). The total volume of mixture was 20 μl . Oligonucleotide primers used for amplified of the exon 2 of BoLA-DRB3 for the first round of the reaction:

HLO-30 (5'-3': TCCTCTCTCTGCAGCACATTTC)
HLO-31 (5'-3': ATTCGCGCTCACC TCGCCGCT)

Five μl of DNA used as a template, regardless of its concentration. Primers used for amplified second round:

HLO-30 (5'-3': TCCTCTCTCTGCAGCACATTTC)
HLO-32 (5'-3': TCGCCGCTGCACAGTGAACTCTC)

The PCR products first round (2 μl) were transferred to a second round. First stage was started from DNA denaturation at 95 °C for 5 min followed by 10 cycles with denaturation (94 °C for 1 min), annealing (62.5 °C for 2 min.) and

elongation (72 °C for 1 min) and a final extension at 72 °C for 7 min. Second stage was started initial denaturation (95 °C for 5 min), was followed by 35 cycles of denaturation (68 °C for 30 sec), and annealing-extension (72 °C for 30 sec and a final extension (72 °C for 7 min). We included in each PCR-round five µL of the last PCR product. It were electrophoresed on 1.5 % agarose gels in order to check the quality and specificity of DNA fragment amplification and control of contamination and self-priming.

PCR products were digested separately with three restriction endonucleases: *RsaI* (fig.), *HaeIII*, *XhoII* (Promega, USA, New England BioLabs and SibEnzim, Russia). The restriction fragments we separated by electrophoresis in a 9 or 12 % agarose gel. Amplification of exon 2 of the gene by means of a PCR followed by the analysis of restriction fragment length polymorphism and comparison of DNA patterns obtained using the three specified restriction endonucleases allows the identification of 54 alleles of the BoLA-DRB3 gene [7, 14, 31].

In order to identify the associations between BoLA-alleles and certain sign, it is necessary to establish the strength of association and the statistical significance between the frequencies of gene carriers in alternative groups of animals. To determine the associations between the alleles of the gene BoLA-DRB3.2 and SCC, the index of relative risk was used (*RR* — relative risk: *a*, *c* — samples with a higher level of SCC from animals having or not having an appropriate allele; *b*, *d* — samples with a lower SCC level from animals having or not having an appropriate allele):

$$RR = \frac{ad}{bc} \quad (1)$$

Pearson's chi-squared test (χ^2) indicates a statistically significant difference between the frequencies of alleles among alternative properties. For two-by-two contingency tables the allele is considered as associated with certain property if the condition $RR \geq 2$ and $\chi^2 > 3.84$ ($P < 0.05$) is satisfied. If the value $RR \leq 0.5$ then the presence of the allele in the animal genotype indicates a close association with the alternative property. In our calculations, the alternative property is value of $SCC = 2 \times 10^5$ cells/cm³, since its excess

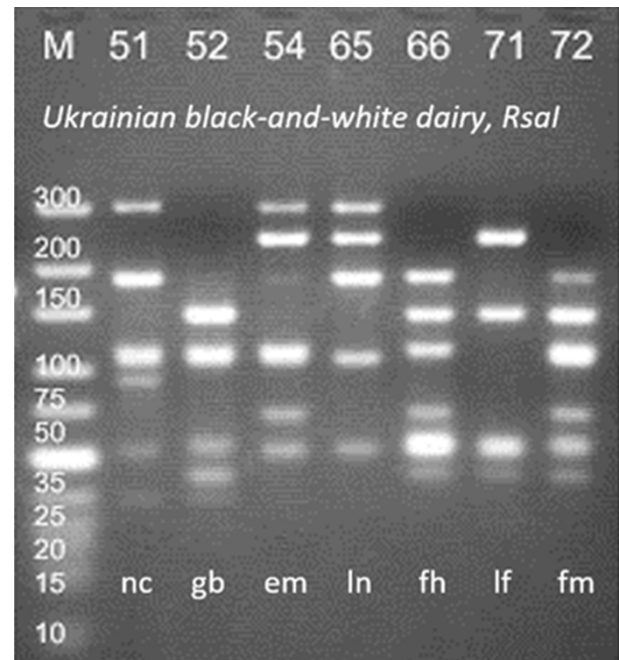


Fig. Restriction analyze of the products amplification of gene BoLA-DRB3 exon 2 with the endonuclease *RsaI*. From above — serial number of cow, beneath — pattern of restriction, M — marker *GeneRuler™* Ultra Low Range DNA Ladder marker (*Fermentas*, Canada).

indicates the infection of the udder [20, 21]. In order to highlight a positive association, relative risk values are set as $1/RR$ with a minus sign.

Test χ^2 makes sense if the sample involves at least 20 animals and the respective conditions are met (*N* — sample size):

$$\begin{aligned} (a+b) \times (a+c) / N &> 5, \\ (a+b) \times (b+d) / N &> 5, \\ (c+d) \times (a+c) / N &> 5, \\ (c+d) \times (b+d) / N &> 5, \end{aligned} \quad (2)$$

Detected associations were refined by a test χ^2 for small samples and Fisher's exact test controlled by Pearson's contingency coefficient (<http://medstatistic.ru/calculators/calchi.html>). In the absence of an allele in the sample, a correction is using the formula of Woolf-Haldane [11, 12].

Experimental studies were performed on two groups of 2–3 lactation cows, which, by analogy, were divided into control (clinically healthy) and experimental (with signs of subclinical mastitis (CM) group of 7 animals in each. Cows of the experimental group injected intrascapiently the Lmanin liposomal drug three times in the affected fourth quarter I with an interval of 24 hours, the first

Table 1

BoLA-DRB3.2 alleles associated with SCC

Allele BoLA-DRB3.2	Allele frequency, P(A)	Relative risk, <i>RR</i>	Test χ^2	Standard error, <i>SE</i>	Test χ^2_{\min} on the limited sample			
					$\frac{(a+b)(a+c)}{N}$	$\frac{(a+b)(a+c)}{N}$	$\frac{(a+b)(a+c)}{N}$	$\frac{(a+b)(a+c)}{N}$
*03	0.06	-2.24 ^P	1.57	0.018	7.77	3.35	57.2	23.8
*04	0.022	-2.52 ^P	1.1	0.011	2.83	1.17	62.2	25.8
*07	0.054	-2.52 ^P	1.1	0.017	7.77	3.83	57.2	23.8
*08	0.06	1.12	0.01	0.018	7.77	4.3	57.2	23.8
*10	0.06	4.73 ^N	1.68	0.018	1.41	0.59	63.6	26.4
*12	0.022	-2.52 ^P	1.1	0.011	4.95	2.28	60.1	25.0
*13	0.038	1.04	0.07	0.014	1.41	0.63	63.6	26.4
*16	0.076	2.83 ^N	1.21	0.02	0.71	0.3	64.3	26.7
22	0.06	-3.43 ^P	3.84 []	0.018	5.65	3.04	59.4	24.7
*23	0.044	7.58 ^{WH}	2.47	0.015	24.1	15.2	41.0	17.0
*24	0.185	1.0	0	0.029	0	0	65.0	27.0
*26	0.027	1.71	0.06	0.012	3.53	1.63	61.5	25.5
28	0.076	-4.14 ^P	6.17 []	0.02	9.89	3.8	55.1	22.8
*32	0.027	4.5 ^{WH}	1.15	0.012	3.53	1.74	61.5	25.5
*36	0.027	0.605	0.48	0.012	3.53	1.52	61.5	25.5
*37	0.027	1.71	0.06	0.012	3.53	1.63	61.5	25.5

Note: absent 15 alleles with frequency lower than 0.02 (total frequency 0.136); P — positive association; N — negative association; WH — Woolf-Haldane correction; * — $P < 0.05$.

10 cm³, and 5 cm³ on the next two days, half of the therapeutic dose was administered prophylactically in the healthy quarter of the mammary gland.

To determine the number of somatic milk cells in the sick animals in the control group, samples of parenchymal milk were taken for the first, third and ninth day of the experiment. Determination of the number of somatic cells was performed directly with the help of Prescott-Breed's calculation method.

Statistical processing performed in the package *Microsoft Excel 2013* with the use of our own programs and integrated customization GenAlEx 6.5 and in the standard package *IBM SPSS Statistics V24.0*.

Results and discussion

In the samples of milk taken from 92 cows the number of somatic cells changed from 84 to 6926 thousand cells/cm³. Among them, only 27 samples of SCC did not exceed the threshold of 200 thousand cells/cm³. Subclinical mastitis had 22 animals. In samples of milk taken from these animals it was found from 876 to 4436 thousand cells/cm³. The SCC value was from 2264 to 6926 thousand cells/cm³ in five cows with clinical mastitis.

In 65 healthy cows the number of somatic cells ranged from 84 to 704 thousand cells/cm³.

Our study established high allele diversity of BoLA-DRB3 gene, characteristic for Ukrainian black-and-white livestock. In the result of 92 blood samples typing, 31 alleles were revealed. The results of the study are shown in table 1.

Dominating alleles that exceed the threshold of 5 % are important for the analysis. 7 of them were detected: *03, *07, *08, *10, *16, *22, *24 and *28. Allele BoLA-DRB 3.2 *24 was the most widespread in the study (18.5 %). 32 cows (34.8 %) were its vectors. Among 57 genotypes, the most frequent variant was *16/*24 (6.5 %). Most genotypes were detected 1–2 times. Six cows had homozygotes (6.5 %): *22/*22 — 3 cases, *10/*10 — 2 cases and *24/*24 — 1 case, in accordance.

The results of the alleles distribution coincide with the data given in literature sources. The allele BoLA-DRB3.2*24 was the most common among purebred Holsteins and Holsteinized herds. In the Holstein herd (Canada) it was found maximum 25 % of it [13]. This allele dominates among the Holstein of Canadian Ontario — 19.2 % [26] and of the American States of Iowa, Wisconsin, Minnesota and Illinois 14.3 % [5]. In the Holstein-

ized breeds, allele *24 was found mostly among the Iranian 19.6 % [16] and the Argentine 15 % [9] cattle. Among different herds of Ukrainian black-and-white dairy breeds, the frequency of this allele was ranged from 6.7 to 18 %. Such distribution is due to the fact that the Holsteins' bloodiness in most herds exceeds 90 % [11]. The dominant alleles discovered in our study had similar positions in other breeds [3, 5, 9, 11, 16, 18, 26, 30].

The main task of this study was to identify the reliable links in the system of "allele — SCC". By the magnitude of the relative risk, significant associations have been found in 10 alleles, 6 of which indicate the connection with the low level of SCC (*03, *04, *07, *12, *22, *28) and 4 — with high content of somatic cells (*10, *16, *23 and *32). The value RR for alleles *22 and *28 are calculated by the amendment Woolf-Haldane, because cow's alleles among selected samples with a low level SCC are absent. This distorts the assessment of the possible association. The strength of association of alleles *22 and *28 in the system "allele — SCC" will be detected when increasing the number of animals tested.

The statistical assessment of detected associations by χ^2 test showed that the significant connection to the low level of SCC had two alleles ($CI = 0.95$): BoLA-DRB3.2*22 ($P(A) = 0.06$; $RR = -3.43$; $\chi^2 = 3.84$) and BoLA-DRB3.2*28 ($P(A) = 0.076$; $RR = -4.14$; $\chi^2 = 6.17$).

Both alleles cannot withstand the test for small samples for test χ^2_{\min} on the limited sample. The condition $(a+b)(a+c)/N > 5$ is not fulfilled; since the number of alleles in milk samples with low SCC from cows that are carriers of the gene is negligible.

To verify the strength of the association, a check has been made by Fisher's exact test controlled by Pearson's contingency coefficient. For the BoLA-DRB3.2*22 allele, the value $P = 0.075 > 0.05$ and $C = 0.2$ (low association force) were obtained. This eliminates the possibility of using it as a marker. For the BoLA-DRB3.2*28 allele, the value $P = 0.0206 < 0.05$ and $C = 0.26$ (average association force) was obtained, suggesting it as a marker that indicates a connection with a low level of somatic cells in milk of cows of Ukrainian black-and-white milk breeds.

Among 92 tested cows that had alleles *28 in genotype, 11 were classified as healthy, and three had mastitis. The average number of somatic cells in healthy cows was 194.9 thousand cells/cm³. All eight cows that had alleles *22 in genotype (3 homozygotes) did not suffer from mastitis. The average SCC in them was 319.7 thousand cells/cm³. This indicates the need to expand research in the direction of identifying associations in the "allele — SCC" system.

Most of authors emphasize the connection between the alleles of gene Bola-DRB3 and SCC, and inflammation of udders. Previously conducted research in identification of associations between the alleles of Bola-DRB 3.2 and the content of somatic cells have a very ambiguous character.

Kelm et al. found the connection between alleles BoLA-DRB3.2*11 and *16, higher number of somatic cells and increased frequency of mastitis [10]. Dietz et al. [5] showed significant correlations between the polymorphism of the BoLA-DRB3 and SCC gene. They think that, the presence of the allele *16 was associated with an increase in the number of somatic cells, whereas allele *22 was associated with low SCC values. In addition, alleles *11, *12 and *23 indicate on the resistance to clinical mastitis [5, 6].

Zanotti et al. reported that by regression analysis, considering DRB3.2 polymorphism, alleles *08 and *22 showed a significant negative effect and allele *16 a significant positive effect on the SCC parameter [34].

Contradictory results were presented by Sharif et al. [26] and Sender et al. [24] who found that allele *16 was associated with a lower level of SCC in milk, and allele *23 with elevated SCC and susceptibility to mastitis.

Rupp et al. found that alleles DRB3.2*03 and *24 were associated with higher AMIR (antibody-mediated immune response) but lower CMIR (cell-mediated immune response) whereas allele *22 was associated with lower AMIR but higher CMIR. Additionally, BoLA DRB3.2*03 and *11 were associated with lower SCC, whereas alleles *22 and *23 were associated with higher SCC. The results of associations between BoLA DRB3.2 and production traits were, in some cases, antagonistic in that BoLA DRB3.2 alleles *11 and *23, which are associated with increased production traits,

were associated with lower and higher SCC, respectively [23].

Pashmi et al. [19] showed a significant correlation between elevated SCC, which reflects an increase in the probability of subclinical mastitis and alleles DRB3.2*08 ($P<0.03$), *22 ($P<0.06$) and *51 ($P<0.08$).

Among the 25 BoLA-DRB3.2 alleles discovered in the Chinese Holstein of South China on the basis of the GLM of SCC (General Linear Model), four (*03, *08, *18 and *26) were associated with lower SCC ($P<0.01$) [33].

In a later study of Baltian et al., the method of classical case-control study design by Fisher's exact test and Woolf-Haldane odds ratio was used to detect the connection between SCC and frequencies of alleles BoLA-DRB 3.2.

In the studied population, significant association was noted with allele BoLA-DRB3.2*23 and *27 ($P<0.05$) with protective and susceptibility effect respectively. In addition, alleles BoLA-DRB3.2*20 and *25 exhibited suggestive association with high SCC ($P<0.1$) [1].

In the study of Oprzadek et al. [17] it was confirmed a decrease in the value of lnSCC for allele *16 (overall effects -0.08 ; $P\leq 0.01$) and increase for allele *23 (overall effects 0.06 ; $P\leq 0.01$) when analyzing all the lactation together. There was also proved the credible influence of ($P\leq 0.01$) allele *24 on phenotypical value SCC in milk [10, 28].

Treatment of subclinical mastitis of cows with the "Limanin" drug led to a decrease in SCC in the milk of experimental animals (table 2).

Table 2

Content of somatic cells in the milk of cows ($M\pm m$, $n=7$)

Indicator	Animal groups	Study period		
		before treatment	3 rd day of treatment	9 th day from the beginning of treatment
SCC*10 ⁻³ cells/ml	control	259,3±40,5		
	experimental	667,9±64,9	573,7±52,08	388,7±44,97

The number of somatic cells in secretion of the mammary glands of the cows of the experimental group decreased on the 9th day in comparison with the 1st day of the experiment ($P<0.01$), indicating the normalizing effect of the components of the study drug and therapeutic effect of this preparation on the content of somatic cells in the milk of cows suffering from subclinical form of mastitis.

The "Limanin" drug is a liposomal antibacterial drug consisting of: novoimanin — extract of St. John's wort, common (*Hypericum perforatum* L.), vitamins A, D₃, E, lecithin, tween. Active for gram-positive bacteria, including *Streptococcus pyogenes* and *Streptococcus agalactiae*. Anti-inflammatory action is due to the presence of flavonoids in the preparation. It has the ability to heal the surface of the wound and stimulates tissue regeneration.

Conclusions

To improve the natural genetic resistance to udder pathogens in succeeding generations

resistance trait SCC are being used in the current breeding programs. The SCC as selection criteria measure the capacity of the cow to resist infection by udder pathogens. It is important to establish a connection between the phenotypic feature and the BoLA system genes.

In the result of allelic polymorphism of Gene Bola-DRB3 study in connection with the content of somatic cells in samples of milk taken from cows of Ukrainian black-and-white dairy breeds, we have established that there is a statistically significant connection between allele *28 and low level of somatic cells. Another allele *22, with a similar connection, had not withstand Fisher's exact test controlled by Pearson's contingency coefficient, although all cows that had this allele in their genotypes had never any form of mastitis.

The allele *28 as SCC marker was not detected in any of the previous studies. Their analysis shows large differences between association of different alleles BoLA-DRB3.2 with SCC, obtained for different breeds and even within the same breed.

The discrepancy of the results is explained by various reasons, such as differences in environmental conditions, peculiarities of bacterial flora, criteria established for diagnostics of mastitis and methods of determination of SCC.

Today, the use of genetic markers related to the content of somatic cells in milk of cows with the subsequent correlation on stability or susceptibility to mastitis is possible only within certain populations, for which a statistically significant relation between the alleles of gene Bola-DRB3 and SCC is established.

Perspectives of the future investigations.

The following studies consist in the expansion of cows research sampling. This will not only clarify the association between the number of somatic cells and the alleles of the gene Bola-DRB3, but also discover the associations in the system “genotype — SCC”. It is necessary to develop scientific-methodical approaches for solution of more complex associations in system “Genotype — SCC — Mastitis”. It is also desirable to extend research on other breeds of Ukrainian dairy cattle.

Research is being conducted to determine the effect of the “Limanin” drug on the number and functional activity of T- and B-lymphocytes in cows suffering from subclinical mastitis.

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